REMARKS

Claims 284-568 were previously pending in this application. New claims 569-1711 have been added in place of former claims 284-568. Accordingly, claims 569-1711 are presented for further examination on the merits.

Applicants acknowledge with appreciation the courtesy extended by Dr. Ardin Marschel at the interviews held on January 27 and May 8, 2000, both of which were attended by Dr. Dean L. Engelhardt, Senior Vice President for the present Assignee and also attended by their undersigned attorney. The presentation of new claims 569-1711 and new information submitted herewith follow the substance and issues discussed at the interviews.

Acknowledgement is made that the Group Art Unit for this application has been changed from the previous Group Art Units 1634 and 1655. Henceforth, any and all future correspondence will be directed to Group Art Unit 1631.

Applicants appreciate the indication in the November 23, 1999 Office Action that the finality of the previous office action was withdrawn pursuant to the provisions of 37 CFR §1.129(a), and that their previous response has been entered.

A new title of the invention has been entered. The new title is believed to be more descriptive of Applicants' claimed invention.

The specification has been amended in two instances. First, disclosure which was previously deleted from the original specification has been re-inserted into the specification. That disclosure spanned over fifty pages (pages 1 through page 52, line 20) and comprised the patent disclosure of Dr. David C. Ward which was filed on April 17, 1981 as U.S. Patent Application Serial No. 06/223,255. Dr. Ward's disclosure culminated in four separate U.S. patents. Because it was incorporated by reference into the present application, the deletion of Dr. Ward's disclosure on February 3, 1995 in the parent application (Serial No. 07/954,772) was thought at that time to be an appropriate step. Upon further r fl ction, however, Applicants and their attorney believe that the re-insertion of Dr. Ward's

disclosure will improve the readability of the present application, thereby aiding future readers in understanding better the present invention being claimed.

Second, the specification has been amended on page 1 (line 1) by the substitution of a new "Reference to Other Related Applications." This new information has been inserted largely in response to the Examiner's astute observation on page 2 of the November 23, 1999 Office Action that a citation and status for the second application filed in the family (Serial No. 06/674,352) had been omitted. This information has now been properly entered, together with the complete information for other related applications and issued U.S. patents.

Commensurate with their broad disclosure, Applicants have substituted new claims 569-1711 in place of former claims 284-568, the latter having been canceled. In order to facilitate the Examiner's review, Applicants' attorney is presently preparing a chart that will cross-reference the new claims against the previously pending claims has been prepared. As soon as it has been completed, Applicants will submit the cross-referencing chart in a supplemental response to the present Amendment.

New claims 569-1711 are directed to the same subject matter as the previously pending and now canceled claims. That is to say, processes are still being claimed for nucleic acid sequencing, nucleic acid detection, chromosomal characterization, and nucleic acid labeling, although the order has been changed from the previous claims.[1]

One aspect now being claimed in the new claims, however, is the use of Applicants' modified nucleotides and "nucleotide analogs, which nucleotide analogs can be attached to or coupled to or incorporated into DNA or RNA, and wherein said one or more modified or labeled nucleotides or nucleotide analogs have been modified or labeled on at least one of the sugar moiety, the sugar analog, the

[1] **Thus, in the revised order of subject matter, new claims 569-1711 are directed to a process for determining the sequence of a nucleic acid of interest (claims 569-1297), a process for detecting a nucleic acid of interest (claims 1298-1472), processes for chromosomal characterization, including determining whether the number of copies of a particular chromosome in a cell is normal or abnormal (claim 1473), identifying a chromosome of interest in a cell containing other chromosomes (claim 1474), identifying a plurality or all of the chromosomes in a cell of interest (claim 1475), and determining the number of chromosomes in an interphase cell of interest (claim 1476), and dependent claims therefrom (1477-1581); and a process for preparing a labeled oligo- or polynucleotide of interest (claims 1582-1699).**

phosphate moiety, the phosphate analog, the base moiety, or the base analog thereof; . . ." The issue of Applicants' use of modified nucleotide analogs was discussed earlier this year at the January 27, 2000 interview. Elaboration and supporting evidence for this aspect of Applicants' claimed invention is discussed *infra*. See this Amendment, page 187, last paragraph, through page 217, first full paragraph.

It should also be noted that a great many multiple dependencies have been eliminated in new claims 569-1711 at the expense of additional dependent claims attached to each independent claim. Furthermore, a whole set of independent claims (1700-1711) have been added which are directed to the aforementioned subject matter in which the one or more signaling moieties comprise a chelating compound or chelating component capable of providing a detectable radioactive signal. The latter set of claims was briefly discussed at the January 27, 2000 interview.

In presenting new claims 569-1711 above, consideration was given to the outstanding issues set forth in the November 23, 1999 Office Action. These issues include:

- the specific localization of modified nucleotides as given in previously pending claim 367[2] (new matter rejection under 35 U.S.C. §112, first paragraph);
- the hybridizing practice as set forth in previously pending detection claim 284[3] (indefiniteness rejection under 35 U.S.C. §112, second paragraph); and
- the hybridization detection process as set forth in previously pending claim 396[4] using detectable protein binding sequences (anticipation rejection under 35 U.S.C. §102(b)).

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[2] Former and now canceled claim 367 recited:
The process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one terminal modified nucleotide.

[3] Former and now canceled claim 284 recited in part: "(a) hybridizing . . . and (b) detecting the presence . . ."

[4] Former and now canceled claim 396 recited:
A process for detecting a nucleic acid of interest in a sample, which process comprises the steps of:

In further detail, the subject matter previously directed to claims 284, 367 and 396 has been omitted from the new claims or drastically altered to address the rejection(s) set forth in the November 23, 1999 Office Action. Referring to former and now canceled claim 367, the subject matter of this claim has been omitted from new claims 569-1711 altogether. In the case of Applicants' nucleic acid detection claims, new claims 1298-1410 all call for specific hybridization between the oligo- or polynucleotide and the nucleic acid of interest. More particularly, in the main detection process claim 1298, the first recited step calls for "(a) specifically hybridizing said nucleic acid of interest in the sample with one or more oligo- or polynucleotides, each such oligo- or polynucleotide being complementary to or capable of hybridizing with said nucleic acid of interest or a portion thereof, . . . In the case of Applicants' nucleic acid detection processes using a protein binding sequence (claims 1411-1472, new independent claim 1411 calls for three steps. First, there is (A) provided (i) an oligo- or polynucleotide having two segments. The first segment (a) is complementary to and capable of hybridizing to a portion of the nucleic acid of interest; and the second segment (b) comprises at least one protein binding nucleic acid sequence. Also provided in step (A) is (ii) a detectable protein which is capable of binding to the protein binding nucleic acid sequence. The second step in claim 1411 is (B) contacting a sample suspected of containing the nucleic acid of interest with the oligo- or polynucleotide (i) and the detectable protein (ii) to form a complex. The third step in the claim requires detecting non-radioactively the presence of the protein in the complex and the nucleic acid of interest.

Entry of new claims 569-1711 is respectfully requested.

Footnote 4 Continued

(a) hybridizing said nucleic acid of interest in the sample with an oligo- or polynucleotide comprising at least one detectable protein binding sequence capable of binding to said nucleic acid of interest; and

(b) detecting the presence of said detectable protein binding sequence, thereby detecting said nucleic acid of interest.

Priority Claim Under 35 U.S.C. §120

As indicated in the opening remarks above, the specification has been amended by inserting on page 2 a new section titled REFERENCE TO OTHER RELATED APPLICATIONS. U.S. Patent Application Serial No. 06/674,352, filed on November 21, 1984 has been cited and its abandoned status has been indicated.

The First Rejection Under 35 U.S.C. §112, First Paragraph

Claim 367 stands rejected for new matter under 35 U.S.C. §112, first paragraph. In the Office Action (page 3), the Examiner stated:

> The specific localization of modified nucleotides as given in instant claim 367 has also not been found as filed and is therefore NEW MATTER. It is noted that generic locations of modified nucleotides does not support a specific specie as claimed in claim 367 of specifically two locations never disclosed together in the instant specification.

As indicated in the opening remarks above, the subject matter of former claim 367 has been omitted from the new claims.[5] Accordingly, the rejection for new matter is believed to have been rendered moot.

In view of the presentation of the new claims, reconsideration and withdrawal of the new matter rejection is respectfully requested.

Before addessing the substantive issues in the November 23, 1999 Office Action, Applicants would like to elaborate on the issue of "nucleotide analogs," "base analogs," "sugar analogs" and "phosphate analogs," as set forth in the new claims. In the Examiner Interview Summary Record dated January 27, 2000, it was indicated that [the Examiner and Applicants' representatives discussed]

[5] Former and now canceled claim 367 was directed to "[t]he process of claim 348, wherein the labeled oligo- or polynucleotide of interest prepared by said incorporating step comprises at least one internal modified nucleotide and at least one terminal modified nucleotide."

. . . "the metes and bounds of nucleotide analogs and self-indicating in the claim language."

In response, Applicants wish to point out that the new claims are directed to processes for sequencing, detection and labeling in which modified nucleotides or nucleotide analogs are utilized in conjunction with non-radioactive labeling and detection (or radioactive detection in the case where chelating compounds or components are employed). Nucleotide analogs are defined in the new claims as those "which can be incorporated into DNA or RNA." An artisan working in the field of nucleic acids, including nucleic acid sequencing and hybridization detection, would appreciate and understand the meaning and extent of the foregoing claim language. Moreover, such an artisan working in the field of nucleic acids would also appreciate and understand from this claim language which members would be included in such nucleotide analogs and which members would be excluded.

The term "nucleotide analog" or equivalent language and examples are disclosed numerous times in the specification.[6] In fact, there are no fewer than 34 references or examples in the specification to nucleotide analogs and these are listed below.[7]

Specification References to Nucleotide Analogs

Description	Page/Line
analogs of dUTP and UTP	Page 1, 10th line from bottom
the analogs must be relatively efficient substrates	Page 7, line 9
5-methylcytosine, and 5-hydroxymethylcytosine	Page 9, 2nd & 3rd lines from bottom

[6] The term "nucleotide analog" naturally embraces nucleotidyl subelements including "sugar analogs," "phosphate analogs," and "base analogs," as recited in the new claims.
[7] It should not be overlooked that the disclosure f the original Ward application, U.S. Patent Application Serial No. 06/225,223, filed on April 17, 1981, was incorporated int the present specification. It may well be that there are additional instances of references to "nucleotide analogs" in Serial No. 06/225,223 that have not been included in the 34 references or examples cited in the present specification and listed here.

Description	Page/Line
thymidine analog	Page 31, line 14
analogs of dUTP and UTP	Page 37, 12th line from bottom
5-hydroxy-methylcytosine (5 HMC)	Page 54, 2nd and 3rd full paragraphs
reacting nucleic acids in the double helical form with alkylating agents as for example benz(o)pyrene diol epoxide or aflatoxin. Under appropriate conditions the N^2 group of guanine, the N^4 group of adenosine or the N^4 group of cytosine are alkylated	Page 54, last paragraph
5-Hydroxymethyl-2'-deoxycytidylic acid	Page 60, Example X
5-(4-aminobutylaminomethyl)-2'-deoxyuridylic acid	Page 61, Example XI
Biotinylated-5-(4-aminobutylaminomethyl)-2'-deoxy-uridylic acid	Page 61, Example XII
5-formyl-2'-deoxyuridine	Page 62, Example XIII
Biotinylated 5-formyl-2'-deoxyuridine	Page 63, Example XIV
Biotinylated 5-amino-2'-deoxyuridine	Page 63, ExampleXV
5-(oxy)acetic acid-2'-deoxyuridine	Page 64, Example XVI
Biotinylated 5-(oxy)acetic acid-2'-deoxyuridine	Page 64, Example XVII
5-hydroxymethyl-2'-deoxycytidine-5'-triphosphate	Page 66, Example XIX

Description	Page/Line
maltotriose nucleotide [maltotriose coupled 5-(3-amino-1-propenyl)-2'-deoxyuridine-5' triphosphate	Page 71, Example XXIII
5-(perfluorobutyl)-2'-deoxyuridine	Page 72, Example XXIV
Tubericydin	Page 72, Example XXV
Toyocamycin	Page 72, Example XXV
Maltotriose coupled 5-(3-amino-1-propenyl)-2'-deoxyuridine-5' triphosphate	Page 75, Example XXXI
Fluorescein coupled 5-(3-amino-1-propyl)-2'-deoxyuridine-5'-triphosphate (AA-dUTP)	Page 76, Example XXXII
5-Bromo-2'-deoxyuridine-5'-phosphate	Page 78, Example XXXV
6-Cyano-2'-deoxyuridine-5'-phosphate	Page 79, Example XXXVII
6-(Methylamino)-2'-deoxyuridine-5'-phosphoric acid	Page 80, Example XXXVIII
Two minor purines 2-Methyladenine 1-Methylguanine	Page 91
Two minor pyrimidines 5-Methylcytosine 5-Hydroxymethylcytosine	Page 91

Description	Page/Line
A nucleotide . . . wherein said base B is 2-methyladenine	Original Claim 71
A nucleotide . . . wherein said base B is 1-methylguanine	Original Claim 71
A nucleotide . . . wherein said base B is 5-methylcytosine	Original Claim 72
A nucleotide . . . wherein said base B is 5-hydroxymethylcytosine	Original Claim 73
A nucleotide . . . wherein said base B is deazaadenine	Original Claim 75
A nucleotide . . . wherein said base B is deazaguanine	Original Claim 76

The specification also describes numerous instances for the attachment, coupling or incorporation of modified nucleotides or nucleotide analogs into DNA or RNA. These instances are set forth below.

Specification References to Attachment, Coupling and Incorporation

Description	Page/Line
nucleotides are modified, such as at the 5 position of pyrimidine or the 7 position of purine, preparatory for the preparation for the preparation therefrom of nucleotide probes suitable for attachment to or incorporation into DNA or other nucleic acid material	Page 52, last paragraph (under "Summary of the Invention")
Oligodeoxyribonucleotides were end-labeled using cytidine-5'-triphosphate and terminal transferase	Page 56, Example IV
Biotin and polybiotinylated poly-L-lysine were coupled to oligoribonucleotides	Page 57, Example V
Formaldehyde coupling of cytochrome C-biotin and polybiotinylated poly-L-lysine to oligodeoxyribonucleotides were carried out	Page 58, Example VII
Ligation of poly dA:poly dT, biotinyl dU to oligo-deoxyribonucleotides was accomplished	Page 60, Example IX
labeling purified DNA [by nick translating] with biotinylated 5-formyl-2'-deoxyuridine	Page 67, Example XX
Lambda DNA was nick translated as described herein with maltotriose coupled to 5-(3-amino-1-propenyl)-2'-deoxyuridine-5' triphosphate and 3H-2'-deoxyadenosine	Page 71, Example XXIII
A DNA prob was ligated to a synthetic DNA composed of repeated sequences of *E. coli* lac operator DNA	Page 77, Example XXXIV

Description	Page/Line
As indicated hereinabove, various techniques may be employed in the practices of this invention for the incorporation of the special nucleotides of this invention into DNA and related structures. One particularly technique referred to herein involves the utilization of terminal transferase for the addition of biotinated dUMP onto the 3' ends of a polypyrimidine or to single-stranded DNA. The resulting product, such as a single-stranded or cloned DNA, which has biotinated dUMP onto the 3' ends thereof, can be recovered . . .	Page 99, second paragraph
These nucleotides are then incorporated into specific nucleic acids using a DNA or RNA polymerase or ligase reaction or a chemical linkage.	Page 101, first paragraph (third sentence)
A nucleotide in accordance with Claim 1 wherein . . . such that when said nucleotide is incorporated into or attached to or associated with a double-stranded deoxyribonucleic acid or double-stranded ribonucleic acid or DNA-RNA hybrid, . . .	Original claim 7
A ribonucleotide in accordance with Claim 143 wherein when said nucleotide is incorporated into or attached to a double-stranded deoxyribonucleic acid or double-stranded ribonucleic acid or DNA-RNA hybrid, . . .	Original claim 145

With specific reference to "nucleotide analog which can be incorporated into DNA or RNA," Applicants also wish to point out that this term was well-known and recognized in the art before the advent of their first patent application filing in June 1982.

The literature, which includes an entire textbook devoted specifically to nucleotide analogs (and actually titled "Nucleotide Analogs") as well as other well-known textbooks by the Nobel Prize winning author and scientist, Dr. Arthur Kornberg of Stanford University, is replete with references to nucleic analogs and more particularly, nucleotide analogs which can be attached to or coupled to or incorporated into DNA or RNA or other nucleic acid or genetic structures or material.

In his 1980 textbook titled Nucleotide Analogs: Synthesis and Biological Function [John Wiley & Sons, Inc., New York, 1980, 288 pages], Professor Dr. Karl Heinz Scheit of the Max-Planck-Institut fur Biophysikalische Chemie in Gottingen, Germany, provided an exquisite account of nucleotide analogs covering almost 300 pages. The nucleotide analog compounds described by Dr. Scheit include nucleotides with modified heterocyclic substituents (base analogs), nucleotides with modified phosphate groups (phosphate analogs) and nucleotides with altered sugar parts (sugar analogs). The subject of nucleotides with modified heterocyclic substituents is covered by Dr. Scheit in Chapter 2 (pages 13-89) while the subjects of nucleotides with modified phosphate groups and altered sugar parts are described in Chapter 4 (pages 96-141) and Chapter 5 (pages 142-194), respectively. A complete copy of Dr. Scheit's book is being submitted as Exhibit 1 in Applicants' Supplemental Information Disclosure Statement concurrently filed with their Amendment.

In his 1974 textbook on DNA Synthesis [W. H. Freeman And Company, San Francisco, Chapter 7, pages 227-228, copy attached to Supplemental IDS as Exhibit 2], Dr. Kornberg described several nucleotide analogs which are incorporated into DNA, RNA or DNA and RNA. These include the following:

Dideoxynucleoside triphosphates	Incorporated into DNA
Arabinosyl nucleoside triphosphates	Incorporated into DNA
Cordycepin triphosphates (3'-deoxy ATP)	Incorporated into DNA and RNA
3'-Amino ATP	Incorporated into DNA and RNA
dUTP	Incorporated into DNA
5-Hydroxyuridine, or 5-aminouridine	Incorporated into RNA
5-Bromouracil	Incorporatedinto DNA
Tubericidin "ATP", Formycin "ATP"	Incorporated into RNA and DNA
Dideoxynucleoside triphosphates	Incorporated into DNA
Arabinosyl nucleoside triphosphates	Incorporated into DNA
Cordycepin triphosphates (3'-deoxy ATP)	Incorporated into DNA and RNA
3'-Amino ATP	Incorporated into DNA and RNA
dUTP	Incorporated into DNA
5-Hydroxyuridine, or 5-aminouridine	Incorporated into RNA
5-Bromouracil	Incorporatedinto DNA
Tubericidin "ATP", Formycin "ATP"	Incorporated into RNA and DNA

In a later textbook DNA Replication published in 1980 [W. H. Freeman And Company, San Francisco, Chapter 12, "Inhibitors of Replication," pages 415-441; copy attached to Supplemental IDS as Exhibit 3], Dr. Kornberg devotes an entire chapter subsection to the subject of nucleotide analogs incorporated into DNA or RNA. In fact, the very title of the Subsection 12-3 beginning on page 423 in Dr. Kornberg's book is "Nucleotide Analogs Incorporated into DNA or RNA." The subsection begins with

> Certain analogs of the nucleoside triphosphates, modified in the sugar or base, are accepted by polymerases for pairing with the DNA template and are incorporated into nucleic acid. . .

On page 423 in DNA Replication, Dr. Kornberg then proceeds to list no less than 16 different nucleotid analogs incorporated into DNA or RNA, including th following:

2',3'-Dideoxy NTPs	5-Iodouracil
Arabinosyl NTPs (araC, araA)	Tubercidin
Cordycepin TP (3--deoxy ATP)	Toyocamycin
3-Amino ATP	Formycin
Uracil dNTP (dUTP)	7-Deazanebularin
5-Hydroxyuridine	2-Aminopurine
5-Aminouridine	2-Aminoadenine (2,6-diaminopurine)
5-Bromouracil	2'-Deoxy,2'-azidocytidine

On the next page (224) in Figure 12-4 which is titled "Nucleotide analogs incorporated into DNA or RNA," Dr. Kornberg lists examples of sugar analogs, base analogs of uridine and thymidine, base analogs of adenine and base analogs of adenosine, including the 11 analogs listed below:

<u>Sugar analogs</u>

2',3'-Dideoxynucleoside

Arabinosylnucleoside

Cordycepin

3'-Aminoadenosine

<u>Base analogs of uridine & thymidine</u>

5-Hydroxy-, 5-Amino-, 5-Bromo-, 5-Iodouracil

5-Hydroxy-, 5-Amino-, 5-Bromo-, 5-Iodothymidine

<u>Base analogs of adenine</u>

2-Aminopurine

2-Aminoadenine

<u>Base analogs of adenosine</u>

Tubericidin

Formycin

Toyocamycin

7-Deazanebularin

Other notable textbooks published before the 1982 filing date of this application have also disclosed nucleotide analogs which can be incorporated into RNA and DNA. By way of example, these include the three books described further below.

Dr. J. N. Davidson of the University of Glascow, Edinburgh, Scotland, published seven editions of The Biochemistry of the Nucleic Acids before his death in 1972. Four of his colleagues in the Department of Biochemistry, University of Glascow, authored an eighth edition published in 1976 and appropriately titled Davidson's The Biochemistry of the Nucleic Acids (8th Edition) [Revised by R. L. P. Adams, R. H. Burdon, A. M. Campbell and R. M. S. Smellie, Academic Press, New York, 1976, copy attached to Supplemental IDS as Exhibit 4].[8]

On pages 298 and 299 of Chapter 11 "Replication of DNA," the authors describe analogs in Subsection 11.7.1 *Base and nucleoside analogues*:

> Some of the artificially produced base analogues are incorporated into RNA and DNA and may have powerful mutagenic effects [296, 301, 302, 304, 308, 309]. Among the most important analogues are the halogenated pyrimidines, and those bases where nitrogen has been substituted for a —CH= group (see Fig. 11.29).

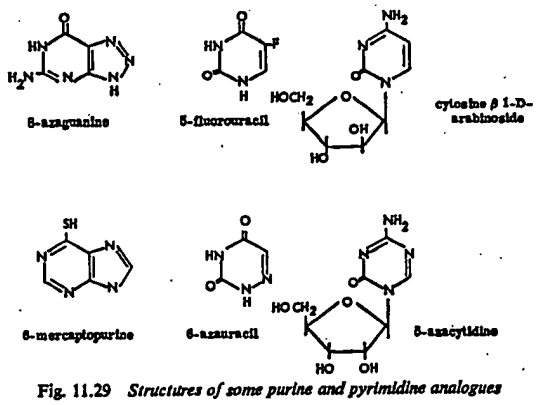

Fig. 11.29 *Structures of some purine and pyrimidine analogues*

[8] Later editions of this work by Adams' group also disclosed the same material on base and nucleoside analogues, including their Tenth Edition published in 1986. Although not reviewed by Applicants' attorney in the course of preparing this Declaration, one would expect that the Ninth Edition of The Biochemistry of the Nucleic Acids published in 1981 and one year before the filing date of this patent application would contain similar if not identical subject matter on base and nucleoside analogues as in the Eighth and Tenth Editions.

On page 299, a second action of these base and nucleoside analogs is described:

> (2) They [base and nucleoside analogs] are themselves, after conversion into nucleotides, incorporated to varying degrees into RNA and/or DNA, although the incorporation may take an abnormal form. Thus 8-azaguanine can be incorporated at the expense of guanine into the RNA of TMV [317] and, to a much larger extent, into the RNA of *B. cereus* [citation omitted]. Only very small amounts are incorporated into the DNA.
>
> 5-Azacytidine is incorporated into RNA but this rapidly interferes with protein synthesis [162, 304]. 5-Azadeoxycytidine is incorporated into DNA, but this renders the cells non-viable [319]. 5-Bromouracil can replace thymine in DNA where it normally base-pairs with adenine. However, in its rare enol-state (which it assumes more readily than does thymine) it may pair with guanine instead of adenine so bringing about the base-pair transition of A-T into G-C. DNA containing 5-bromouracil instead of thymine is very susceptible to breakage at light-induced bromouracil dimers [320] (see below).
>
> The D-arabinosyl nucleosides are effectively analogues of deoxyribonucleosides (e.g. cytosine β-D-arabinoside is incorporated in place of deoxycytidine into DNA where it causes chain termination or a marked reduction in the rate of further chain extension [320-322].

Another significant text disclosing nucleotide analogs and published a year earlier in its English translation earlier than Davidson's Biochemistry of the Nucleic Acids was written by Peter Langen [Antimetabolites of Nucleic Acid Metabolism: The Biochemical Basis of Their Action, with Special Reference to their Application in Cancer Therapy, Gordon and Breach, New York, English edition translated from the German by Dr. Thomas A. Scott, 1975, pages 143-187, copy attached to Supplemental IDS as Exhibit 5]. The second section of Langen's book covers more than twenty-five pages and is devoted to pyrimidine analogs and their nucleosides, purine analogs and their nucleosides, nucleoside analogs with modified sugar components and nucleotide analogs with modified phosphate groups. Almost 70 such analogs are described by Langen, many of which are incorporated into DNA or RNA, as set forth in the quoted passages below.

> (1)
> 2. 5-Fluorouridine 5'-phosphate is incorporated into RNA in plac of uridine 5'-phosphate. This incorporation and its biological consequences ar discussed in d tail on page 71.
>
> [page 144]

(2)
The preparation and the elucidation of the mechanism of action of 5-trifluoromethyl-2'-deoxyuridine are due to HEIDELBERGER et al. It is incorporated into DNA in place of thymidine.

[page 146]

(3)
Owing to the similarity in the atomic radii of bromine (1.95 Å), iodine (2.16 Å) and the methyl group (2.00 Å), 5-bromo- and 5-iodo-2'-deoxyuridine are metabolized by the cell in the same way as thymidine and incorporated into DNA.

[page 147]

(4)
Up to 100% of the thymine of DNA can be replaced by analogues.

[page 147]

(5)
In normal tissues with a high rate of mitosis, e.g. bone marrow and intestinal epithelium, the rate of incorporation of halogenated thymine analogues into DNA is higher than in most types of tumour.

[page 148]

(6)
5-Chlorouracil, like 5-bromouracil and 5-iodouracil, was recognized very early as an inhibitor of bacterial growth [773; 775], but has not been extensively studied. Considering the size of the atomic radius of chlorine, it should be possible to incorporate 5-chlorouracil into both DNA and RNA (see p. 6).

[page 148]

(7)
5-Aminouracil is incorporated into DNA [876] and into RNA [1681].

[page 149]

(8)
5-Methylamino-2'-deoxyuridine causes a transitory increase in the growth of thymine auxotrophs of *E. coli*, which is followed by cell death [1807]. It is possibly incorporated into DNA.

[page 149]

(9)
The activity of 5-hydroxyuracil is similar to that of 5-aminouracil and its inhibitory action on bacterial growth was discovered very early [773; 775]. The preparation and use of the riboside and deoxyriboside was reported later [97]. The riboside is phosphorylated in the cell to the triphosphate, which inhibits RNA-polymerase and is also incorporated into RNA [1491; 1682].

[page151]

(10)
5-Ethyluracil is incorporated into the DNA of thymine auxotrophs of *E. coli* and of T3 phage cultured on these strains (see p. 70). Since the base is a poor substrate for thymidine phosphorylase [1361], a much higher incorporation is obtained after applying the 2'-deoxyriboside.
[page 151]

(11)
The cytostatic and virostatic activity of 2-thiouracil are due to its incorporation into RNA (see p. 87).
[page 154]

(12)
To a limited extent, the 5'-triphosphate of 4-thiothymidine can replace thymidine 5'-triphosphate in the *E. coli* polymerase system. After the attachment of a few residues of 4-thiothymidine 5'-triphosphate, the polymer primer is effectively "poisoned" and the reaction stops.
[page 154]

(13)
5-Azacytidine is incorporated into DNA and RNA and thereby disturbs the function of the nucleic acids (see pp. 71 and 90).
[page 158]

(14)
6-Azathymine was discovered very early as an inhibitor of bacterial growth [453; 1403; 1404; 1415]. Although it is incorporated into DNA in place of thymine (see p. 71), this is not responsible for the inhibition of growth [1403; 1404].
[page 159]

(15)
2-Fluoroadenosine 5'-triphosphate inhibits RNA-polymerase and, at the same time, some of the analogue is possibly incorporated into RNA [1611].
[page 167]

(16)
2-Aminopurine is incorporated into DNA. The incorporation and its consequences are discussed more fully on page 72. . . The 5'-triphosphate, which is necessary for the incorporation into DNA, is formed by the phosphorylation of 2'-deoxyriboside.
[page 167]

(17)
Tubercidin is an antibiotic from *Streptomyces tubercidicus*. It is converted intracellularly into the mono-, di- and triphosphates [6; 397] and incorporated into the DNA and RNA (see p. 89). Tubercidin 5'-di (or tri) phosphate is a substrate for ribonucleotide reductase [1730], as would be expected from its incorporation into DNA.

[page 170]

(18)
Toyocamycin is an antibiotic from *Streptomyces*. It is phosphorylated intracellularly to the 5'-mono, di- and triphosphates, and incorporated into DNA and RNA [1731] (see p. 89). As in the case of tubercidin, the 5'-di, or 5'-triphosphate is a substrate for ribonucleotide reductase, thus fulfilling the requirement for incorporation into DNA [1730].

The incorporation of this compound into nucleic acids and polynucleotides is discussed more fully on page 89. . .

[pages 170 & 171]

(19)
8-Azaguanine was known as early as 1945 as a growth inhibitor of *E. coli* [1470]. Investigations by KIDDER et al [737; 946] with *Tetrahymena gelii* and by SKIPPER et al [1236] with tumours showed that the antimetabolite is incorporated into nucleic acids. This was the first observation of the incorporation of an unnatural base into nucleic acids.
. . . The consequences of the incorporation of 8-azaguanine into RNA are discussed more fully on page 86. The incorporation of 8-azaguanine into DNA is either very low or non-existant (e.g. [1684]. . .

[page 171]

(20)
3-Deoxadenosine (cordycepin) is an antibiotic from *Cordyceps militaris*. It is phosphorylated intracellularly to the mono-, di- and triphosphates [975; 976; 1610] and is incorporated into DNA [353] and RNA [1609]. The inhibition of RNA-polymerase by 3'-deoxyadenosine [526; 977; 1614] is due to the incorporation of the inhibitor into the chain ends of RNA; further extension of the chain is then prevented by the absence of the 3'-hydroxyl group.

[page 176]

(21)
3'-Deoxy-3'-aminoadenosine, which is also an antibiotic from *Cordyceps militaris*, inhibits RNA-polymerase and is possibly incorporated into RNA [1610; 1784].

[pag 176]

(22)
2',3'-Dideoxyadenosine inhibits the multiplication of *E. coli* [1213]. The inhibition appears to be due to the incorporation of inhibitor into DNA (see p. 72), which prevents further extension of the chain.
[page 177]

(23)
3'-Deoxythymidine may also be included here. This compound is not itself biologically active, because it is not phosphorylated in the cell. The chemically prepared 5'-triphosphate is incorporated into DNA in the cell free system, where it inhibits further chain extension.
[page 178]

(24)
L-Nucleosides are taken up by mammalian cells, but not by bacteria [1815a]. L-Adenosine is phosphorylated in the mammalian cell to the 5'-monophosphate and, to a much less extent, to the 5'-diphosphate [873]. It is probably also incorporated into RNA.
[page 183]

(25)
. . . Adenosine 5'-[methylenediphosphonyl] phosphate can replace adenosine 5'-triphosphate in the isolated RNA-polymerase system [1635]. . .
. . . In some reactions, e.g. the formation of the complex that initiates peptide elongation on the ribosome, guanosine 5'-triphosphate can be replaced by its analogue [Guanosine 5'-[methylenediphosphonyl] phosphate], but the subsequent reactions, which involve the decomposition of the guanosine 5'-triphosphate, are inhibited.
[page 187]

Many scientific articles have also been published on the subject of nucleotide analogs, including those which are incorporated into DNA or RNA. By way of examples, the eight (8) articles listed below, all of which were published before 1982, disclose such nucleotide analogs as those recited in the claims of this patent application. Included below are brief quotations from these articles of references or examples of nucleotide analogs which can be incorporated into DNA or RNA.

A. Darlix et al., "Analysis of Transcription *in Vitro* Using Purine Nucleotide Analogs," Biochemistry 10:1525-1531 (1971) [copy attached to Supplemental IDS as Exhibit 6].

(1)
Formycin and 8-deazaguanine, structural analogs of adenosine and guanosine, respectively (Robin *et al.* (1945); Koyana *et al.* (1966), inhibit nucleic acid synthesis in bacterial and mammalian cells (Shapiro *et al.* (1950); Chantrenne (1964); Hori *et al.* (1964); Ward *et al.* (1969)). The analogs resemble their normal counterparts in H-bonding potential and in the formation of base pairs with complementary template residues, and they are also effectively incorporated into RNA *in vivo* and *in vitro* (Shapiro *et al.* (1950); Smith and Mathews (1957); Mathews (1957); Brockman *et al.* (1959); Caldwell *et al.* (1966); Ward *et al.*(1969).

[page 1525, left column, 1st ¶]

(2)
. . . Under these conditions the relative yield of chains initiated by the **purine nucleotide analogs** [8-azaGTP and FTP] was decreased still further.

[page 1526, right column, penultimate ¶]

(3)
(c) INITIATION KINETICS AT LOW **NUCLEOTIDE ANALOG** CONCENTRATIONS.

[page 1527, right column, last ¶]

(4)
*III. Effect of **Purine Analogs** on Chain Release.*

[page 1530, left column, 1st full ¶]

(5)
Discussion
The results presented in this paper provide additional insights into possible biochemical mechanisms of action of **base analogs** and identify some steps in transcription of DNA which may be susceptible to exogenous controls.

Our data show that individual stages in transcription can be differentiated by their response to the **base analogs**; . . .

[page 1530, left column, last two ¶s]

B. Geider, K., "DNA Synthesis in Nucleotide-Permeable *Escherichia coli* Cells: The Effects of Nucleotide Analogues on DNA Synthesis," European Journal of Biochemistry 27:554-563 (1972) [copy attached to Supplemental IDS as Exhibit 7].

(1)
The effects of nucleotide analogues on DNA synthesis were studied in nucleotide-permeable *Escherichia coli* cells.

[page 554, abstract]

(2)

RESULTS

Inhibition of DNA Synthesis by 22'.3'-Dideoxyribosylthymine Triphosphate

Log phase *E. coli* H512 was harvested and made permeable to nucleotides by shaking the 100-fold concentrated cell suspension with ether as described in Materials and Methods. DNA synthesis in the presence of deoxyribonucleoside triphosphate is strongly inhibited by ddTTP, a dTTP analogue lacking the 3'-hydroxyl group. Increasing concentrations of ddTTP reduce the deoxyribonucleoside triphosphate incorporation into *E. coli* DNA more and more (Fig. 1, lower curves) . .

[page 556, left column, 1st full ¶]

C. Darlix and Fromageot, "Restriction of gene transcription by nucleotide analogs," Biochimie 56:703-710 (1974) [copy attached to Supplemental IDS as Exhibit 8].

(1)

In this communication, the influence of the purine nucleoside triphosphate analogs on RNA chain termination induced by rho was investigated, using T7 phage DNA as a template . . .

[page 703, left column, 1st ¶]

(2)

RESULTS

EFFECTS OF PURINE ANALOGS ON *in vitro* T7 DNA TRANSCRIPTION.

[page 703, lower right column]

(3)

. . . So both purine analogs tend to restrict gene transcription in the presence of rho, albeit FTP less dramatically than 8-azaGTP.

[page 706, lower left column]

(4)

. . . Double standed(sic) ribopolymers containing either analog were synthesized with *E. coli* RNA polymerase to directly test the influence of these purine analogs on RNase III activity . . .

[page 706, right column, 2nd ¶]

(5)

Finally, the restriction of gene transcription by nucleotide analog might be exploited to achieve selective expression of particular regions of genomes *in vivo* and/or *in vitro*.

[page 709, right column, last ¶]

D. Marcus, F., "Inhibition of Fructose 1,6-Biphosphatase by 9-ß-D-Arabinofuranosyl 5'-Monophosphate," Cancer Research 36:1847 (1976) [copy attached to Supplemental IDS as Exhibit 9].

(1)
There has been an increased interest in the study of nucleoside analogs as potential therapeutic agents in the treatment of neoplastic diseases (for references, see Ref. 1) The field is also expanding to nucleotide analogs . . .

[page 1847, 1st ¶]

(2)
Other close AMP [adenosine monophosphate] analogs (*i.e.* the 5'-phosphates of deazaadenosine, formycin, isoadenosine, tubercidin) may also inhibit fructose 1,6-bisphosphatase, thereby altering the regulation of carbohydrate metabolism. Adenine nucleotide analogs could also affect the key regulatory glycolytic enzyme phosphofructokinase, as recently mentioned by Bloch[3] in his studies with tubercidin.

[page 1847, 2nd ¶]

E. Simoncsits and Tomasz, "A New Type of Nucleoside 5'-Triphosphate Analogue: P1-(Nucleoside 5'-) P1-Amino-Triphosphates," Tetrahedron Letters 44:3995-3998 (1976) [copy attached to Supplemental IDS as Exhibit 10].

Compounds of type 2 as nucleoside 5'-triphosphate analogues containing chemically modified, chiral α-phosphorus atoms may be important from the point of view of different chemical and enzymatic studies . . .

[page 3997, last ¶]

F. Chladek et al., "Synthesis and Properties of Nucleoside 5'-Phosphoazidates Derived from Guanosine and Adenosine Nucleotides: Effect on Elongation Factors G and Tu Dependent Reactions," Biochemistry 16:4312-4319 (1977) [copy attached to Supplemental IDS as Exhibit 11].

(1)
ABSTRACT: A new type of nucleoside poly(5'-phosphate) analogue, nucleoside 5'-phosphoazidate, with an azido group on the terminal phosphate of GTP, ATP, GDP, GMP, and AMP, has been synthesized . . .

[page 4312, top of th page]

(2)
We have chosen as a probe the photolabile azido group, placed at the terminal phosphate of GTP and GDP. In this paper, we describe our initial studies of the synthesis and properties of these new compounds, the nucleoside 5'-phosphoazidates. We also report on the behavior of these analogues of GTP and GDP . . .
[page 4312, right column, 2nd full ¶]

(3)
Results
Synthesis and Proof of Structure of Nucleoside 5'-Phosphoazidates IIIa-f. Michelson (1964) reported the synthesis of various nucleoside anhydrides as well as nucleotide anhydrides by anion displacement on p1-nucleoside 5'-p2-diphenylpyrophosphates. It was found that a variety of anions of acids weaker than diphenylphosphoric acid could attack esterified pyrophosphates of the general type II (Scheme II) . . . We have used the method of Michelson (1964) for the synthesis of the novel nucleotide analogues, nucleoside 5'-phosphoazidates (IIIa-f). . .
[page 4315, lower right column, through page 4316, 1st ¶]

G. Piperno and Alberts, "An ATP Stimulation of T4 DNA Polymerase Mediated via T4 Gene 44/62 and 45 Proteins," Journal of Biological Chemistry 253:5174-5179 (1978) [copy attached to Supplemental IDS as Exhibit 12].

(1)
. . . In this report we use nucleotide analogues to demonstrate that this polymerase stimulation requires hydrolysis of the β,γ-phosphate bond of ATP.
[page 5174, abstract]

(2
In this study we use rATP and dATP analogues to demonstrate that the above stimulation of T4 DNA polymerase by the accessory proteins requires the hydrolysis of ATP or dATP . . .
[page 5174, right column, last line, through
page 5175, left column, first two lines]

H. Reha-Krantz et al., "Bacteriophage T4 DNA Polymerase Mutations That Confer Sensitivity to the PPi Analog Phosphonoacetic Acid," Journal of Virology 67:60-66 (1993) [copy attached to Supplemental IDS as Exhibit 13].

(1)
. . . As found for herpes simplex virus DNA polymerase, T4 mutations that altered sensitivity to phosphonoac tic acid also altered sensitivity to nucleotide analogs . . .
[page 60, abstract]

(2)
. . . As was observed for HSV DNA polymerase studies, T4 DNA polymerase mutations that altered PAA sensitivity also affected interactions with nucleotide analogs; . . .
[page 60, left column, last ¶]

(3)
Sensitivity to ddNTPs and other nucleotide analogs. HSV DNA polymerase mutant strains isolated for the ability to replicate in the presence of PAA or PFA are often less sensitive to the antiviral action of nucleotide analogs such as acyclovir (16, 27) and are cross resistant to 2',3'-dideoxynucleoside triphosphates (ddNTPs) (9)
[page 63, right column, 1st full ¶]

(4)
. . . but it is predicted from HSV DNA polymerase studies that altered sensitivities to nucleotide analogs will also be detected for at least some of the mutants.
[page 63, right column, 2nd full ¶]

(5)
Mutant DNA polymerase respond in a consistent pattern to PPi and nucleotide analogs. As discussed above, wild-type HSV DNA polymerase is sensitive to PAA and mutants that are PAA sensitive are cross resistant to the nucleotide analogs acyclovir and ddNTPs but hypersensitive to PAA (44) . . . While wild-type T4 DNA polymerase is highly resistant to PAA, mutants that are PAA sensitive are cross resistant to nucleotide analogs.
[page 63, right column, last line, through page 64, left column, 1st two lines]

(6)
. . . Derse et al. (9) suggested that these conflicting observations can be explained if PAA and nucleotide analogs have similar inhibitory mechanisms: . . . If either of these models is correct, it is predicted that DNA polymerase mutations that alter PPi and nucleotide analog interactions would also alter translocation . . .
[page 64, left column, 1st full ¶]

(7)
Identification of a DNA polymerase active center. Single amino acid substitutions at several sites in T4 DNA polymerase and in other family B DNA polymerases altered interactions with both PPi and nucleotide analogs, . . . Gibbs et al. (16) proposed from their studies of HSV DNA polymerase that none of the regions identified in the drug sensitivity studies is the sole binding site for either PPi or dNTPs because amino acid substitutions that alter PAA or PFA sensitivity also alter interactions with nucl otide analogs and/or aphidicolin.
[page 65, left column, 1st full ¶]

Sugar Analogs

Scientific articles have also been published on the subject of sugar analogs, including those sugar analogs which can be incorporated into DNA or RNA. Listed below are five (5) articles including brief descriptions or references to the term "sugar analog."

A. Birch and Lee, "Structural Functions and Taste in the Sugar Series: The Structural Basis of Bitterness in Sugar Analogues," Journal of Food Science 41:1403-1407 (1976) [copy attached to Supplemental IDS as Exhibit 14].

(1)
. . . This report re-examines some of the published information relating to sweetness and bitterness in sugars and their analogues in an attempt to explore molecular characteristics which may be responsible for eliciting the bitter response.

[page 1403, left column, 1st ¶]

(2)
MATERIALS & METHODS
ALL SUGARS and their analogues described or referred to in this report were either chromatographically pure crystalline materials obtained from British Drug Houses (Chemicals, Poole, Dorset) or known and novel compounds synthesized by classical carbohydrate techniques as previously reported.

[page 1403, left column]

(3)
. . . With the sugars and analogues, panelists were asked to place a few milligrams of each substance on the tongue and to comment whether . . .

[page 1403, left column, last three lines]

(4)
RESULTS & DISCUSSION
CONSIDERABLE NUMBERS of the sugar, model glycosides and analogues have already been examined . . .
[page 1403, right column]

B. Lartey and Derechin, "Preparation and Study of a Fluorescent Sugar Analog: Competitive Inhibitor of Yeast Hexokinase," Preparative Biochemistry 9:85-95 (1979) [copy attached to Supplemental IDS as Exhibit 15].

> . . . this investigation may open a new direction for active center research since a number of additional fluorescent sugar analogs, each exhibiting a distinct binding property, can be synthesized using similar procedures to those employed here. The potential usefulness of such compounds . . . follows from the known facts . . . and (ii) each subunit in the dimer carries one sugar binding site which, depending on experimental conditions, will bind one sugar (or sugar analogue) or another up to a maximum of two such ligands per dimer[17].
> [page 93, 2nd ¶]

C. Roberts and Hayes, "Effects of 2-deoxy D-glucose and other sugar analogues on acid production from sugars by human dental plaque bacteria," Scandinavian Journal of Dental Research 88:201-209 (1980) [copy attached to Supplemental IDS as Exhibit 16].

(1)
Key words: acid production; dental plaque; sugar; sugar analogues.
[page 801, under the abstract]

(2)
In the present study, rates of acid production were measured using aliquots of the same plaque suspension so as to compare acid formation from a range of sugars and sugar alcohols and to investigate the usefulness of 2DG and other sugar analogues as inhibitors of acid production . . .
[page 801, right column]

D. Kortnyk et al., "CMP and CMP-sugar analogs as inhibitors of sialic acid incorporation and glycoconjugates," Eur. J. Med. Chem. - Chimica Therapeutica 15:77-84 (1980) [copy attached to Supplemental IDS as Exhibit 17].

CMP and CMP-sugar analogs have been synthesized and evaluated as inhibitors of CMP-N-acetylneuraminic acid : glycoprotein sialyltransferase (E.C. 2.4.99) as well as L 1210 murine leukemia cells in culture and *in vivo* as anticancer agents . . .

[page 77, abstract]

E. Keppler et al., "Uridylate trapping, induction of UTP deficiency, and stimulation of pyrimidine synthesis *de novo* by D-galactosone," Biochemical Journal 206:139-146 (1982) [copy attached to Supplemental IDS as Exhibit 18].

(1)

D-galactosone (D-*lyxo*-2-hexosulose) was among the first sugar analogues synthesized (Fischer, 1889). In contrast to D-glucosone, this C-2 modified D-galactose analogue was previously not considered to be toxic (Bayne, 1952; Bayne & Fewster, 1956). Earlier work has established that the toxicity of some sugar analogues, including D-galactosamine, is due to their interference with uracil nucleotide . . .

[page 139, left column]

(2)

Our present investigation with a D-galactose-metabolizing hepatoma cell line (Keppler, 1974*a*) and in rat liver have revealed that -galactosone can act as a most powerful uridylate-trapping agent, inducing a higher rate of pyrimidine synthesis *de novo* than any of the sugar analogues studied previously . . .

[page 139, right column, last ¶, through page 140, first three lines]

(3)

Discussion

D-galactosone acts as a powerful uridylate-trapping sugar analogue in hepatoma cells and liver . . .

[page 145, left column]

(4)

The interference of uracil nucleotide metabolism induced by D-galactosone indicates that this C-2-modified hexose analogue can be highly effective in D-galactose-metabolizing tissues and cells such as liver and hepatoma . . . This property may be most useful in experimental chemotherapy where uridylate-trapping hexose analogues serve to induce short-term UTP deficiency . . .

[page 146, left column, last paragraph]

Phosphate Analog (or equivalent language)

The term "phosphate analog" or equivalent language also appeared in the scientific literature before the June 1982 filing date of the present application. The two articles listed below both refer to "phosphate analog" or equivalent langugage.

A. Yang and Metzler, "Pyridoxal 5'-Phosphate and Analogs as Probes of Coenzyme-Protein Interaction," Methods in Enzymology 62:528-551 (1979) [copy attached to Supplemental IDS as Exhibit 19].

(1)
Reconstitution of Apoenzymes with Pyridoxal-P, Pyridoxamine-P, and Analogs
A variety of derivatives and analogs of the coenzymes are available. The coenzymes may be methylated on the ring nitrogen, on phenolic oxygen, or on the phosphate group.
[page 540, bottom of page, through page 541, 1st line]

(2)
. . . For example, from Fig. 2 we conclude that the 430-nm substrate aldimine is more strongly stabilized with the 5-ethylphosphonate analog than with pyridoxal-P. . .
[page 550, last line, through page 551, first two lines]

B. Stridh et al., "The Effect of Pyrophosphate Analogues on Influenza Virus RNA Polymerase and Influenza Virus Multiplication," Archives of Virology 61:245-250 (1979) [copy attached to Supplemental IDS as Exhibit 20].

(1)
Analogues of pyrophosphate have been tested as inhibitors of influenza virus RNA polymerase activity in cell-free assays. . . .
[page 245, abstract]

(2)
Several types of structures have been suggested as inhibitors of influenza virus multiplication (12). One type. which does not seem to have been systematically investigated, is analogues of pyrophosphate.
[page 245, 2nd full ¶]

(3)
Analogues of pyrophosphate were tested for inhibition of the virion associated influenza virus RNA polymerase under assay conditions essentially as described by BISHOP *et al.* (2).
[page 245, 3rd full ¶]

In another scientific article published two years before this application was first filed, the term "sugar phosphate analogue" is disclosed and used. This article by Stoeckler et al. ["Human Erythrocyte Purine Nucleoside Phosphorylase Reaction with Sugar-Modified Nucleoside Substrates," Biochemistry 19:102-107 (1980); copy attached to Supplemental IDS as Exhibit 21] provides at least twelve (12) descriptions relating to the use of the term "sugar-phosphate analog." These twelve descriptions are listed below.

(1)
The kinetic paramters (K_m and V_{max}) of sugar-modified analogues of inosine and guanosine have been determined . . . The sugar phosphate analogue, 5-deoxyribose 1-phosphate, was synthesized from 5'-deoxyinosine with immobilized PNP, and its presence was verified . . . The adenosine versions of the 5'-modified analogues were also found to react with adeosine deaminase, albeit at 1% of V_{max}.

[page 102, abstract]

(2)
Further, the remarkably high activity (10-15 units/ml. of cells) of PNP in human erythrocytes may degrade various nucleoside analogues of chemotherapeutic potential in transit through the blood stream to the desired site of action.

[page 103, left column, 1st ¶]

(3)
The present report documents the activity of human erythrocytes PNP with a number of sugar-modified adenosine analogues by reaction with calf intestinal adenosine deaminase.

[page 103, 1st full ¶]

(4)
. . . Adenosine analogues were converted to their inosine counterparts by deamination . . .

[page 103, right column, 1st ¶]

(5)
The phosphorylation and synthesis of guanosine and its analogues were monitored directly at 258 nm . . .

[page 103, right column, 1st full ¶]

(6)
Many of the inosine analogues examined were prepared from the respective adeosine analogues through deamination by reaction with calf intestinal adenosine adeaminase. Since these adenosine analogues varied greatly in reactivity with ADA, it was necessary to adjust the ADA concentration and the reaction time to the particular adenosine analogue. In each case, formation of the respective inosine analogue was monitored spectrophotometrically by measuring the decrease in absorbancy at 265 nm. The adenosine analogues (1.0-5.0 nM) and 1-10 units of calf intestinal ADA were incubated in 2-mL reaction volumes for time periods necessary for complete conversion to inosine analogues, i.e., a few seconds to several hours.
[page 104, left column, 1st ¶]

(7)
Immobilized Enzymes for the Synthesis of 5-Deoxyribose 1-Phosphate
For use in the synthesis of inosine analogues from adeosine analogues and in the preparation of 5-deoxyribose 1-phosphate, PNP and adenosine deaminase were immobilized on agarose by a modification of published procedures (Pharmacia Fine Chemicals, 1976) . . .
[page 104, left column, 2nd ¶]

(8)
Results
Activity of Sugar-Modified Analogues with PNP.
Table I presents the kinetic parameters of the compounds studied . . .
[page 104, lower right column]

(9)
Substrate Activation
. . . As shown in Figure 2, similar substrate activation occurred at high concentrations of the sugar-modified analogues 5'-deoxy- and 2',5'-dideoxyinosine . . .
[page 105, left column, 1st full ¶]

(10)
Substrate Activity with Adenosine Deaminase The inosine analogues no. 3-11 of Table I were produced from the respective adenosine analogues, which were not active with PNP. When the adenosine analogues were incubated with calf intestinal adenosine deaminase, it was observed that those modified at C(5') were deaminated to completion but at extremely low rates . . .
[page 105, right column, 1st full ¶]

(11)

Although there have been brief reports of the interactions of various sugar-modified nucleoside analogues with PNPs from several sources, there have been no detailed analyses of the kinetic parameters of this class of analogues with human erythrocytic PNP . . . Therefore, since many of the nucleoside analogues under consideration in this paper and elsewhere have potential as chemotherapeutic or immunosuppressive agents, it is important to appreciate that one may not be able to predict the reactivity of such analogues with human PNP on the basis of the studies performed with enzymes from other species.

[page 106, left column, last ¶, through right column, 1st ¶]

(12)

. . . A nucleoside analogue with good reactivity with deoxyguanosine kinase but low activity for PNP might form cytotoxic analogue nucleosides in T lymphocytes without prior degradation by PNP. Also, if potent inhibitors of PNP could be identified and coadministered with analogues of deoxyguanosine, e.g., ß-D-2'-deoxythioguanosine, enhanced intracellular formation of analogue deoxynucleotides, e.g., deoxythioGTP, might be achieved. The most potent PNP inhibitor reported to date, the inosine analogue formycin B, has a relatively high Ki value (1×10^{-4} M) but is capable of inhibiting the phosphorolysis of 6-thioinosine in intact erythrocytes (Sheen et al., 1988) . . . Since large quantities of this enzyme are readily purified from human erythrocytes and high activity may be bound in a stable form, i.e. ~50 units/g of Sepharose, even sugar-modified nucleosides that display low Vmax values with PNP may be converted to the respective pentose 1-phosphates through the use of prolonged incubation times. These novel pentose 1-phosphates may be used for the synthesis of new families of nucleosides through prolonged incubation with agarose-bound PNP and base analogues such as 6-thioguanine, 6-selenoguanine, and 8-azaguanine.

[page 106, left column, last ¶, through page 107, 1st seven lines]

That those persons in the art of nucleic acid technology, including sequencing, detection and chromosomal characterization, would appreciate and understand the meaning and the limits conveyed by the claim language (nucleotide analog which can be attached to or coupled to or incorporated into DNA or RNA) is also bolstered by the U.S. patent literature. A brief survey of this literature shows that numerous U.S. patents have been issued with claim language such as "nucleotide or nucleotide analog," or its equivalent "oligonucleotide or oligonucleotide analog." Listed below are sixteen (16) patents, together with reference to one or more claims reciting such language.

Inventor(s)	U.S. Pat. No.	Language/Claim Nos.
Ryser et al.	4,847,240 (Exhibit 22 IDS)	claim 21 (drug nucleotide or nucleotide analog)
Schwartz et al.	5,212,059 (Exhibit 23 IDS)	claim 1 (said probes comprise a selective sequence of 10 to 100 nucleotides or nucleotide analogs)
Banker et al.	5,643,730 (Exhibit 24 IDS)	claim 11 (in the presence of one or more radiolabeled nucleotides or detectable nucleotide analogs)
Usman et al.	5,652,094 (Exhibit 25 IDS)	claim 3 (The enzymatic nucleic acid molecule . . . wherein W and Y together comprise at least one nucleotide or nucleotide analogue . . .)
Eigen et al.	5,807,677 (Exhibit 26 IDS)	claim 23 (A method . . . carried out using nucleotides or nucleotide analogs . . .)
Liu et al.	5,914,230 (Exhibit 27 IDS)	claims 10 & 34 (. . . each of said linking groups comprising from 0 to 40 nucleotides or nucleotide analogs)
Wright et al.	5,998,383 (Exhibit 28 IDS)	claims 1, 3, 5 & 6 (A synthetic antisense oligonucleotide comprising at least twelve nucleotides or nucleotide analogues)
Pagano et al.	5,242,906 (Exhibit 29 IDS)	claims 1-2 (An oligonucleotide or oligonucleotide analog consisting essentially of . . .)

Inventor(s)	U.S. Pat. No.	Language/Claim Nos.
Ecker et al.	5,591,600 (Exhibit 30 IDS)	claims 1-7 (method . . . oligonucleotide or oligonucleotide analog . . .)
Anderson et al.	5,591,720 (Exhibit 31 IDS)	claim 1 (An oligonucleotide or oligonucleotide analog having . . .)
Cook et al.	5,614,617 (Exhibit 32 IDS)	claims 1-3 & 6-7 (oligonucleotide or oligonucleotide analog comprising . . .)
Baker	5,643,780 (Exhibit 33 IDS)	claims 21-23 & 25-27 (oligonucleotide or oligonucleotide analog)
Rahman et al.	5,665,710 (Exhibit 34 IDS)	claim 1 (method of encapsulating an oligodeoxynucleotide or an analog thereof) & claim 3 (. . . separated from oligodeoxynucleotide or oligodeoxynucleotide analog)
Ecker et al.	5,736,294 (Exhibit 35 IDS)	claims 1-6 (method . . . oligonucleotide or oligonucleotide analog)
Crooke et al.	5,811,232 (Exhibit 36 IDS)	claim 1 (An oligonucleotide or oligonucleotide analog) & claim 2 (method . . . oligonucleotide or oligonucleotide analog) & claim 3 (kit . . . oligonucleotide or oligonucleotide analog)
Ecker et al.	5,874,564 (Exhibit 37 IDS)	claims 1-15 (oligonucleotide or oligonucleotide analog)

Inventor(s)	U.S. Pat. No.	Language/Claim Nos.
Martin et al.	5,891,468 (Exhibit 38 IDS)	claim 13 (composition . . . oligonucleotide or oligonucleotide analog)

The information contained in the preceding thirty pages and attached Exhibits 1-38 illustrate the state and knowledge of the art at the time this application was first filed in 1982. Those engaged in the field of nucleic acid technology, including processes for nucleic acid sequencing, nucleic acid detection and chromosomal characterization, would clearly have understood not only the meaning but the metes and bounds of the subject matter covered by the claim language "nucleotide analog which can be attached to, coupled to or incorporated into DNA or RNA."

Before addressing the rest of the issues at hand, Applicants would like to bring to the Examiner's attention three (3) U.S. patents and claims directed to nucleic acid detection and sequencing processes. In these patents, practically no limitation with respect to the nucleotidyl components -- either in the nucleic base or the sugar moiety -- were imposed or required. These patents are listed below together with an exemplary claim.

Smith et al., U.S. Patent No. 5,821,058, licensed to Applied Biosystems, Inc./Perkin-Elmer, issued October 13, 1988, based on a priority filing date of January 16, 1984 (or later depending upon the subject matter by various C-I-P applications [copy attached to this Amendment as Exhibit 1]

Claim 28 of ABI/Elmer Perkin's '058 Patent recites:

> A method of determining the sequence of a polynucleotide which comprises:
>
> providing polynucleotide fragments tagged with chromophores or fluorophores, wherein the chromophores or fluorophores are distinguishable from others by their spectral characteristics;
>
> resolving the polynucleotide fragments by electrophor sis; and
>
> detecting the resolved fragments by means of the chromophore or fluorophores, and thereby determining the sequence based on the polynucleotide fragments detected.

Prober et al., U.S. Patent No. 5,332,666, assigned to Du Pont of Delaware, issued July 26, 1994, based on priority filing date of July 2, 1986 (or June 12, 1987) [copy attached to this Amendment as Exhibit 2]

Claim 1 of DuPont's Prober '666 Patent recites:

> A process of DNA sequence analysis by chain elongation comprising reacting a template of DNA contained in an appropriate sequencing vector, a primer, a polymerase, a first mixture of DNA nucleotides or their analogs, and a second mixture of radiation omitting reporter-labeled chain terminators corresponding to the DNA molecules or their analogs to produce fragments of DNA having reporters covalently attached to their 3'-terminal residues introduced by chain termination events, and analyzing the fragments so produced for the presence of reporters, thereby identifying the DNA sequence.

Middendorf et al., U.S. Patent No. 4,729,947, licensed exclusively to Li-Cor, Inc: of Lincoln, Nebraska, issued March 8, 1988, based on priority filing date of March 29, 1984 [copy attached to this Amendment as Exhibit 3]

Claim 1 of Li-Cor's Middendorf '947 Patent recites:

> A method for sequencing DNA comprising the steps of:
> preparing a multiplicity of identical DNA strands;
> marking the DNA strands on one end with biotin;
> dividing the DNA strands into at least four batches;
> preparing at least one additional batch of DNA strands with known lengths to be electrophoresed as a time base;
> randomly cleaving at least some of the DNA strands in a first batch at adenine bases by a chemical treatment to form an adenine-strand batch;
> randomly cleaving at least some of the DNA strands in a second batch at guanine bases by a chemical treatment to form a guanine-strand batch;
> randomly cleaving at least some of the DNA strands in a third batch at cytosine bases by a chemical treatment to form a cytosine-strand batch;
> randomly cleaving at least some of the DNA strands in a fourth batch at thymine bases by a chemical treatment to form a thymin - strand batch;

applying samples from each of the four batches after cleaving at their respective bases to four identical channels of gel electrophoresis apparatus;

applying at least one time base source of DNA of known length strands to at least one additional channel positioned among the aforementioned four channels whereby a reference time of electrophoresing may be obtained from said channel;

electrophoresing the DNA strands through a gel slab so that bands of more mobile strands, each of which has substantially uniform length strands, are fully resolved while the less mobile strands to be later formed into bands are unresolved in a continuous process such that at least ten percent of the bands are fully resolved and electrophoresed through the gel while the less mobile strands are yet unresolved into bands in the gel; whereby a plurality of adenine-strand bands is formed in a first channel in an order corresponding to the length of the strands; a plurality of guanine-strand bands is formed in a second channel in an order corresponding to the length of the strands; a plurality of cytosine-strand bands is formed in a third channel in an order corresponding to the length of the strands; and a plurality of thymine-strand bands is formed in a fourth channel in an order corresponding to the length of the strands;

attaching fluorescent markers to avidin whereby the fluorescently labeled avidin is combined with the biotin markers at the end of the DNA strands;

moving the fluorescently-marked bands in sequence through a medium;

scanning said bands with laser having a narrow bandwidth substantially
comforming to the optimum absorption spectrum of the fluorescent markers;

pulsing the laser light with pulses of shorter duration than three nanoseconds during a first period of time;

detecting the fluorescent emission from the markers across a narrow selective band of wavelengths conforming substantially to the optimum emission spectrum of the markers during a second period of time; said second period of time beginning at least fifty nanoseconds from the start of its corresponding pulse of laser light and terminating at a time no greater than one hundred fifty nanoseconds from the start of the pulse of the laser light; and

identifying and recording the sequence of the bands in each of the channels so as to indicate the DNA sequence.

Because each of the three above-listed patents bear a priority filing date well after Applicants' June 23, 1982 priority filing date, they have not been included in the Supplemental Information Disclosure Statement being submitted concurrently with this Amendment. N vertheless, Applicants respectfully invit the Examiner to review and consider these patents.

The Second Rejection Under 35 U.S.C. §112, First Paragraph

Claims 284-568 stand rejected under 35 U.S.C. §112, first paragraph, as the disclosure is enabling only for claims limited to a scope of covalent attachment sites of the cited "Sig" moiety to bases of nucleic acids wherein said sites are either the N^2 of guanine, the N^6 of adenine, the N^4 of cytosine, the C^6 of uracil, or the N^7 of adenine. In the Office Action (pages 3-5), the Examiner stated:

> A thorough review of the disclosure as filed has revealed that the chemistry by which nucleic acid bases may be modified so as to attach a "Sig" moiety only is disclosed for the above four attachment sites within the scope of claims 284 etc. For example, the instant disclosure does not discuss in any way the preparation of N-1 or N-3 modified purines or N-3 or C-2 modified pyrimidines. It is noted that claims 284 etc. are already limited in that certain other, non-base, attachment sites on purines, pyrimidines, and deazapurines are not within the scope of the claims for the at least one modified base in probes used in the claimed methods. It is also noted that certain generalized labeling methods are instantly disclosed such as the formaldehyde coupling of cytochrome C as a bridge between biotin and a nucleic acid molecule on page 58 but that such generalized labeling of a nucleic acid probe lacks both instant disclosure as well as predictability as to where the attachment site is on the probe and therefore fails to predictably form attachments as instantly claimed and thus is deemed to fail to enable the broad scope of specific base modifications of the instant claims. Ruth is herein cited as summarizing the lack of knowledge at the time of the instant filing regarding the preparation of nucleic acid hybridization probes which contain a signalling moiety. The earliest disclosure of said summary of Ruth is 2/22/83 which is the filing date of the earliest parent thereof and which is also less than a year after the filing date of the instant application. This therefore summarizes the lack of broad hybridization probe preparatory knowledge even after the instant filing date. Ruth summarizes the preparatory knowledge for signal moiety containing labeled probes in column 1, line 43, through column 3, line 45. As cited therein nucleic acid hybridization probes may be prepared either chemically or enzymatically. Enzymatic synthesis using nick translation is discussed wherein certain base modifications have been incorporated into probes but limited in use due to several factors. One of these factors is that only certain modifications may be incorporated by enzymes. Ward et al.(P/N 4,711,955) summarize the factors that were viewed as limitations on modified nucleotides in column 6, line 36, through column 7, line 17, and thereinafter discuss specific base modifications with detailed and lengthy chemical steps. Ruth at column 3, lines 26-45, also summarizes that chemical synthesis has not been disclosed in the prior art as incorporating modified or reporter group containing nucleotides. Further consideration of Ruth reveals that specific base modifications are therein disclosed such as at column 10, line 57, through column 20 which are accomplished via a

> lengthy series of detailed reactions including the masking and unmasking of reactive side groups to prevent unwanted modifications. Ruth and Ward et al. are deemed representative of those skilled in the art at about the time of the instant filing date of the instant disclosure.

The Examiner's remarks continued on pages 5-6:

> In summary, those skilled in the art at the time of filing of the instant invention viewed the preparation of signal moiety containing nucleic acid probes as lengthy and detailed procedures that were discussed as being accomplished only for certain specific base modifications. It is noted that Ruth or Ward et al. only disclose base modifications at the following sites: C-8 of purines and the C-5 of pyrimidines, N^6 of adenosine, and N^2 of guanosine, and N^4 of cytosine, and C-7 of 7-deazapurines. This documents the lack of enablement of most specific base modifications without detailing lengthy preparatory procedures for those skilled in the art at the time of the instant filing date. Therefore it is deemed undue experimentation to prepare base modified nucleic acid hybridization probes wherein the site of base modifications is other than the N^2 of guanine, the N^6 of adenine, the N^4 of cytosine, or the C-6 of uracil within the scope of instant claims 240 etc. It is again noted that the instant claims are limited so that base modifications at the C-8 of purines, the C-5 of pyrimidines, and the C-7 of 7-deazapurines are not within their scope. Applicants have supplied several references in their submission, filed 9/14/99, regarding the enablement of non-Ward positions. These references have been reviewed and are persuasive that the N-7 position on the adenine base is defined in the art in such a way as being utilizable in such nucleobase labeling. The Zn labeling references, however, attach Zn at the site where purine bases attach to either ribose or deoxyribose and thus is not enabling for labeling hybridization probes as required in the instant disclosure.
>
> The enablement rejection is respectfully traversed.

At the outset, Applicants appreciate the indication from the Examiner in the above-quoted rejection that the N-7 position on the adenine base has been deemed enabled. In order to summarize the issue with respect to base labeling, Applicants have listed those base positions which are enabled, together with those base positions which are still at issue, and other base positions which are not at issue having already been deemed enabled (the so-called "Ward non-disruptive" base positions).

BASE POSITIONS (Enabled)	Pyrimdines	Purines
	N^4 of cytosine	N^2 of guanine
	C6 of uracil	N^6 of adenine
		N^7 of adenine

BASE POSITIONS (At Issue)	Pyrimidines	Purines
	N^1 (sugar attachment)	N^9(sugar attachment)
	C^2	N^1
	N^3	N^3

BASE POSITIONS (Enabled but Not At Issue - "Ward")	Pyrimidines	Purines
	C5	C8
		C7 of 7-deazpurines

As acknowledged by the Examiner in the instant rejection, Applicants submitted twelve (12) references in their May 1, 1999 Communication and an additional five (5) references in their September 7, 1999 Communication. These references totaling 17 in number included the following:

N-1 Position in Purines

Montgomery, J. H. and H. Jeanette Thomas, "4-Amino-7-β-D-Ribofuranosyl-7H-Imidazol[4,5-*d*]-v-Triazine(2-Aza-Adenosine) The Synthesis of 2-Azapurine Nucleosides from Purine Nucleosides Accomplished via Ring Opening Followed by Reclosure with Nitrous Acid," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 118, pages 681-685 [copy attached to Supplemental IDS as Exhibit 39].

Shibaev, V. N. and S. M. Spiridonova, "1-Methyladenosine-5'-(α- -Glucopyranosyl Pyrophosphate): Methylation of Adenosine Derivatives," in Synthetic Procedures in Nucleic Acid Chemistry, Volume I, Werner Zorbach and R. Stuart Tipson, Editors, Interscience Publishers, New York, 1968, No. 14, pages 461-462 [copy attached to Supplemental IDS as Exhibit 40].

N-3 Position in Purines

Thomas, H. Jeanette and J. A. Montgomery, "3-Benzylpurines," in Synthetic Procedures in Nucleic Acid Chemistry, Volume I, Werner Zorbach and R. Stuart Tipson, Editors, Interscience Publishers, New York, 1968, No. 10, pages 28-30 [copy attached to Supplemental IDS as Exhibit 41].

N-7 Position in Purines

Yamamoto et al., "Adenine-N-oxide produced from adenine with gamma-rays and its binding to SH protein," J. Radiation Research 21:239-247 (1980) [copy attached to Supplemental IDS as Exhibit 42].

Rhaese, H. J., "Chemical Analysis of DNA Alterations: III. Isolation and Characterization of Adenine Oxidation Products Obtained from Oligo- and Monodeoxyadenylic Acids Treated with Hydroxyl Reactions," Biochimica et Biophysica Acta 166:31-326 (1968) [copy attached to Supplemental IDS as Exhibit 43].

Taylor, M. R., "Metal Binding to Nucleic Acid Constituents. The Crystal Structure of Trichloroadeniniumzinc(III)," Acta Crystallogr. B29:884-890 (1973) [copy attached to Supplemental IDS as Exhibit 44].

Walker et al., "The Interaction of H+, Zn2+, and Cu2+ With Adenine and Guanine," Australian Journal of Chemistry 26:2391-2399 (1973) [copy attached to Supplemental IDS as Exhibit 45].

Srinivasan et al., "X-Ray Crystal Structure of Zinc-Adenine and Zinc-Guanine Complexes," J. Chemical Society D24:1668-1669 (1970) [copy attached to Supplemental IDS as Exhibit 46].

C-2 Position in Purines

Kaneko et al., "8,2-Anhydrides of Purine -8-Thiol Nucleosides (or of Purine 2'-Thionucleosides): Synthesis of 8,2'-Anhydronucleosides of Purine-8-thiol [or of 8,2'-Anhydro-(2'-thionucleosides)] by use of Diphenyl Carbonate as the Cyclizing Agent," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 103, pages 395-399 [copy attached to Supplemental IDS as Exhibit 47].

C-6 (keto) Position in Purines

Robins, M. J. and G. L. Bason, "6-Chloro-9-(2-Deoxy-β-D-Erythro-Pentofuranosyl)Purine from the Chlorination of 2'-Deoxyinosine: Direct Replacement of the 6-Oxo Group by a Chlorine Atom in a Purine 2'-Deoxynucleoside: Stabilization of the Glycosyl Bond Towards Cleavage by Acid," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 104, pages 601-606 [copy attached to Supplemental IDS as Exhibit 48].

Zemlicka, J. and J. Owens, "6-Chloro-9-β-D-Ribofuranosylpurine: A Versatile Intermediate in the Synthesis of Purine Ribonucleosides," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 106, pages 611-614 [copy attached to Supplemental IDS as Exhibit 49].

N-3 Position in Pyrimidines

Kochetkov et al., "3-Methyluridine 5'-Phosphate: Methylation of Uridine Derivatives with Diazomethane: Phosphorylation of Nucleosides with Pyrophosphonyl Chloride," in Synthetic Procedures in Nucleic Acid Chemistry, Volume I, Werner Zorbach and R. Stuart Tipson, Editors, Interscience Publishers, New York, 1968, No. 152, pages 497-499 [copy attached to Supplemental IDS as Exhibit 50].

Zemlicka, J., "3-Methyl-6-Azauridine [4-Methyl-2-β-D-Ribofuranosyl-AS-Triazin-3,5-(2H,4H)-Dione]: Alkylation of a Nucleoside Antimetabolite by Use of N,N-Dimethylformamide Dimethyl Acetal," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 78, pages 451-453 [copy attached to Supplemental IDS as Exhibit 51].

C-2 Position in Pyrimidines

Piskala, A. and F. Sorm, "1-β-D-Ribofuranosyl-δ-Triazine-2,4-(1H,3H)-Dione (5-Azauridine): Direct Synthesis of a 5-Azauridine Ribonucleoside by the Fisher-Helferich Procedure," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 79, pages 455-459 [copy attached to Supplemental IDS as Exhibit 52].

C-6 Position in Pyrimidines

Poverenny et al., "Immunological Approaches to DNA Structure Investigation - I: Immunological Identification of the Product of Cytosine Modification with Bisulfite and *O*-Methylhydroxylamine Mixture," Molecular Immunology 16:313-316 (1979) [copy attached to Supplemental IDS as Exhibit 53].

Visser, D. W. and P. Roy-Burman, "5-Hydroxyuridine 5-Phosphate Derivatives: Substitution Reactions at the Pyrimidine Ring of Nucleotides," in Synthetic Procedures in Nucleic Acid Chemistry, Volume I, Werner Zorbach and R. Stuart Tipson, Editors, Interscience Publishers, New York, 1968, No. 151, pages 493-496 [copy attached to Supplemental IDS as Exhibit 54].

Cadet, J., "1-(2-Deoxy--β-D-Erythro-Pentopyranosyl)Uracil and Its α-D Anomer: Acid-Catalyzed Isomerization of the Glycosyl Group in 5,5-Dibromo-2'-deoxy-5,6-dihydro-6-hydroxyuridine," Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Part Two, Dr. Leroy B. Townsend and Dr. R. Stuart Tipson, Editors, John Wiley & Sons, New York, 1978, No. 55, pages 311-315 [copy attached to Supplem ntal IDS as Exhibit 55].

Applicants wish to respectfully point out that any of the non-Ward base modification positions still at issue and embraced by the new claims could have been utilized in nucleobase labeling. Moreover, based upon a reading of Applicants' specification taken with the knowledge in the art, nucleotides and nucleotide analogs labeled in such non-Ward base modification positions could have been usefully attached or coupled to an oligo- or polynucleotide, particularly for example, by terminal labeling or even coupling or ligation, both of which are fully disclosed and described in their specification. Such a means of attachment or coupling would not necessarily be prevented by the fact that the site of modification, labeling, attachment, coupling, or ligation otherwise involved a site where the base would normally be attached to a sugar moiety, for example, a ribose, deoxyribose, a dideoxyribose, or any sugar moiety or sugar analog for that matter. In fact, it is clear that any of Applicants' claimed modified nucleotides and nucleotide analogs could be attached, coupled or ligated terminally to an oligo- or polynucleotide.

Applicants respectfully point out that modifications at the N1 and N9 positions of pyrimidine and purines, respectively, were also known in the art in 1982. Thus, nucleobases, including Applicants' claimed base analogs, could have been modified in the N1 and N9 positions as shown by the following fourteen (14) references, each of which was published before 1982.

N9 Position in Adenine

Kondo et al., "Functional Monomers and Polymers, 77[a]: On the Synthesis and Polymerization of Acryloylamino Derivatives of Nucleic Acid Bases," Makromol. Chem., Rapid Commun. 1:303-306 (1980) [copy attached to Supplemental IDS as Exhibit 56]. See, e.g., Structure **2** on page 304.

Nair et al., "Utility of Purinyl Radicals in the Synthesis of Base-Modified Nucleosides and Alkylpurines: 6-Amino Group Replacement by H, Cl, Br, and I," Journal of Organic Chemistry 45:3969-3974 (1980) [copy attached to Supplemental IDS as Exhibit 57]. See, e.g., Structure **6** on page 3970.

Fujii et al., "Purines. XXI.1) Synthesis of Adenine 1-Oxides Carrying an Allylic Side Chain at the 9-Position," Chem. Pharm. Bull. 28:3443-3446 (1980) [copy attached to Supplemental IDS as Exhibit 58]. See, e.g., Structure **1** on page 3444.

Hiraoka, K. and T. Yokoyama, "Syntheses and characterization of polymers containing nucleic acid bases," Int. J. Biolog. Macromolecules 1:50-54 (1979) [copy attached to Supplemental IDS as Exhibit 59]. See, e.g., adenine bases substituted in 1-position with CH_2CH_2OH, CH_2CH_2Br, etc. in Figure 2 on page 52.

Zeleznick, L. D., "6-Amino-9-(4-Dimethylaminobutyl)-9*H*-Purine," in Nucleic Acid Chemistry: Improved and New Synthetic Procedures, Methods and Techniques, Drs. Leroy B. Townsend and R. Stuart Tipson, Editors, John Wiley & Sons, Inc., 1978, pages 17-18 [copy attached to Supplemental IDS as Exhibit 60]. See, e.g., Structure 3 on page 17.

N9 Position in Guanine

Watson, A. A., "Purine *N*-Oxides. LVII. 9-Hydroxyhypoxanthine, Xanthine, and Guanine," Journal of Organic Chemistry 39:2911-2916 (1974) [copy attached to Supplemental IDS as Exhibit 61]. See, e.g., Structures 12 and 13 on page 2913.

N1 Position in Cytosine

Helfer et al., "Selective Alkylation and Aralkylation of Cytosine at the 1-Position," Journal of Organic Chemistry 46:4803-4804 (1981) [copy attached to Supplemental IDS as Exhibit 62]. See, e.g., Structure 3 on page 4803 and Table I for the 1-substituted cytosine compounds (R = methyl, ethyl, propyl, butyl, allyl and benzyl).

Hosmane, R. S. and N. J. Leonard, "Simple Convenient Synthesis of 1-Methylcytosine," Synthesis 2:118-119 (1981) [copy attached to Supplemental IDS as Exhibit 63]. See, e.g., Structure 4 on page 118 (1-methylcytosine).

Hayashi et al., "*N*-Alkylation of Cytosine and Its Nucleosides with Trialkyl Phosphates," Bull. Chem. Soc. Japan 53:277-278 (1980) [copy attached to Supplemental IDS as Exhibit 64]. See, e.g., Structure IIa-c (a: R = Me, b: R = Et, c: R = allyl) on page 277.

N1 Position in Uracil

Kondo et al., "Synthesis of Sulfonic Acid Derivatives of Purine and Pyrimidine," Synthetic Communications 10:267-271 (1980) [copy attached to Supplemental IDS as Exhibit 65]. See, e.g., Structure III on page 268.

Pitha, J. and P. O. P. Ts'o, "N-Vinyl Derivatives of Substituted Pyrimidines and Purines," Journal of Organic Chemistry 33:1341-1344 (1968) [copy attached to Supplemental IDS as Exhibit 66]. See, e.g., Structures **1** (R=OH), **3** (R=Cl) and **5** (R=$OCOCH_3$) on page 1342 and also **1-Vinyluracil** under **Experimental Section** on page 1343.

N1 Position in Thymine

Inaki et al., "Synthesis of Poly-L-Lysine Containing Nucleic Acid Bases," in Modification of Polymers, Charles E. Carraher, Jr. and Minoru Tsuda, Editors, ACS Symposium Series 121, American Chemical Society, Washington, D.C., 1980, pages 359-370 [copy attached to Supplemental IDS as Exhibit 67]. See, e.g., Structure (*11*) on page 365 and structure preceding it on the same page.

N9 Position in Adenine, N1 Position in Cytosine and Thymine

Shimidzu et al., "Synthesis of Cationic Mononucleotide Analogs and Their Interaction with Polynucleotide and Polynucleotide Analogs," Bulletin of the Chemical Society of Japan 52:3362-3365 (1979) [copy attached to Supplemental IDS as Exhibit 68]. See, e.g., the upper four structures in Fig. 1 on page 3362.

In summary and conclusion then, at the time the present application was first filed in 1982, a skilled artisan could have modified, without undue experimentation, Applicants' nucleotides and nucleotide analogs in the non-Ward base positions described in the 30 references listed above (Exhibits 39-68). Having modified Applicants' nucleotides and nucleotide analogs in these ways, the skilled artisan could then have attached, coupled, ligated or incorporated their modified nucleotides and nucleotide analogs into oligo- or polynucleotides.

In view of the foregoing remarks and submitted exhibits, Applicants respectfully request reconsideration and withdrawal of the second rejection und r the first paragraph of 35 U.S.C. §112.

The Third Rejection Under 35 U.S.C. §112, First Paragraph

Claims 284-328, 331, 337-372, 376-395, 402-405, 407, 408, 410, 412, 413, 415, 416, 418, 419, 421, 422, 424, 425, 427, 428, 430, 431, 433, 434, 436, 437, 439, 440, 442, 443, 445, 446, 448, 449, 451, 452, 454, 455, 457, 458, 460, 461, 463, 464, 466, 467, 469, 470, 472, 473, 475, 476, 478, 479, 481, 482, 484, 485, 487,488, 490-495, 497, 498, 500, 501, 503, 504, 506, 507, 509-515, 517-519, 522, 523, 528, and 530-568 stand rejected under 35 U.S.C. §112, first paragraph, as the disclosure is enabling only for claims limited to "SM" moieties which are either ribose or deoxyribose. In the Office Action (pages 7-8), the Examiner stated:

> It is noted that claim 284, lines 13-15, cite "PM" attachment points but does not therein limit the "SM" moiety to the above sugar types. Thus, the scope of "SM" is only presently limited in claims 284 etc. to being a "furanose moiety" which is much broader in scope than that of ribose or deoxyribose. It is noted that there is no instant discussion as to how to practice the synthesis of nucleotides with "SM" moieties other than that of ribose or deoxyribose. It is noted that in order to broadly practice sugar moieties usage both the synthesis of "PM" attachment is required as well as the "Sig" attachment. Additionally hybridization between the nucleic acid of interest and the oligo- or polynucleotide must still be permitted. No guidance whatsoever has been instantly set forth directed to accomplishing this broad sugar moiety practice other than that directed to ribose or deoxyribose sugars which permit hybridization via specific conformations in nucleic acid polymers. It is noted additionally that the numerous examples given in th specification do not include any sugar practice other than ribose or deoxyribose. In the above scope rejection directed to base labeling practice the need for detailed and lengthy procedures to enable the person skilled in the art to prepare nucleotide analogs as well as their incorporation into polymers is summarized. These disclosures include complex chemical protection requirements including those directed to sugar side group protection as well as considerations such as whether enzymes would recognize and incorporate nucleotides into polymers or not as well as other considerations as discussed above. Thus, it is deemed undue experimentation to practice nucleotide compound and polymers containing these compounds without such detailed and lengthy procedural guidance. In summary, such detailed and lengthy guidance is instantly set forth only for "SM" practice directed to ribose or deoxyribose and it is deemed undue experimentation to practice "SM" moieties other than ribose and deoxyribose given the limited instant disclosure.

The rejection for the disclosure being of limited enablement is respectfully traversed.

It is believed that the use of the term "sugar moiety" in the present claims is sufficiently enabling with respect to Applicants' use of their modified nucleotides and nucleotide analogs in processes for nucleic acid sequencing, detection and chromosomal characterization. The skilled artisan could practice, without undue experimentation, Applicants' claimed invention, using species of sugar moieties beyond the ribose and deoxyribose set forth in the rejection, which also could have easily included dideoxyribose. As in the case of many modified bases and base analogs, other species of sugar moieties could be used in tailing or terminal labeling procedures, or otherwise attached or coupled to an oligo- or polynucleotide through ligation or coupling procedures. It is reasonable to expect that such other species of sugar moieties are embraced and enabled in the present claims.

Beyond that, Applicants would like to point out that many U.S. patents have been issued in which the term "sugar moiety" is recited in claims directed to nucleic acids, oligonucleotides, polynucleotides, and the like, thus establishing at least the presumption of validity with respect to the patentability of the instant processes in which "sugar moiety" is recited. Among these U.S. patents are the following eight documents offered for purposes of illustration[9]:

U.S. Pat. No.	Inventor(s)	Claim(s)/Terminology
6,054,440 [Exhibit 4]	Monia et al.	claim 7 (. . . oligonucleotide comprises at least one modified sugar moiety)
5,837,860 [Exhibit 5]	Anderson et al.	claim 2 (said nucleic acid molecule is modified at a 5' carbon of a sugar moiety of the nucleic acid molecule)

[9] **Because each of these eight patents were filed after Applicants' June 23, 1982 priority filing date, none has been included in the Supplemental Information Disclosure Statement being submitted concurrently herewith. Thus, copies have been provided as exhibits attached directly to this Amendment, as listed.**

U.S. Pat. No.	Inventor(s)	Claim(s)/Terminology
5,837,860 (continued)	Anderson et al.	claim 3 (said nucleic acid molecule is modified at a 3' carbon of a sugar moiety of the nucleic acid molecule)
5,801,235 [Exhibit 6]	Pari	claim 2 (The oligonucleotide . . . further comprising . . . at least one modified sugar moiety . . .)
5,691,141 [Exhibit 7]	Koster	claim 25 (. . . modified with . . . attached to the sugar moiety of a 5'-terminal nucleotide . . .)
5,593,840 [Exhibit 8]	Bhatnagar et al.	claim 4 (The process . . . wherein the modification is to the nucleotide sugar moiety)
5,547,835 [Exhibit 9]	Koster	claim 24 (. . . nucleic acid fragments is modified with . . . attached to one or more sugar moieties of nucleotides)
5,543,507 [Exhibit 10]	Cook et al.	claim 1 (A cross-linked nucleic acid comprising . . . a first bond means located on a sugar moiety of said first nucleotide . . .)
5,486,603 [Exhibit 11]	Buhr	claim 5 (The oligonucleotide . . . in which the analog is selected from . . . a modified sugar moiety, . . .)

In view of the foregoing remarks and submitted exhibits, Applicants respectfully requ st r consideration and withdrawal of the third rej ction under 35 U.S.C. §112, first paragraph.

The Rejection Under 35 U.S.C. §112, Second Paragraph

Claims 284-375, 381-384, 396-400, and 402-568 stand rejected for indefiniteness under 35 U.S.C. § 112, second paragraph. In the Office Action (pages 8-9), the Examiner stated:

> Claim 284, part (b), cites the detection of the presence of "oligo- or polynucleotides which have hybridized to said nucleic acid of interest" but is vague and indefinite when considered in view of part (a) of the claim. Said part (a) cites the practice of "hybridizing ... " without any selectivity or specificity directed to preventing hybridization to nucleic acids that are not the "nucleic acid of interest". Thus, such "permitting" practice is reasonably interpreted as inclusive of all levels of stringency including conditions where hybridization is permitted to not only "nucleic acid of interest" but also to other nucleic acids that may be only 90% complementary, 70% complementary, or even only 20% complementary, etc. to the "oligo- or polynucleotide" cited in part (a). With this broad complementarity practice possible within the scope of part (a), what is meant by applicants' citation of the detecting practice of part (b)? Do applicants mean that selectivity or specificity is to be practiced at the detection step and not at the hybridization step? This suggests that the detecting step is not just a detecting step but is also inclusive of some selection practice. Such a selection practice is not given in step (b) as presently worded. It is noted that the commonly performed practice of a hybridization assay is to control the hybridization step, herein step (a) rather than step (b), so as to be selective as desired. Then the detection step is only directed to the detection of a signal which is then indicative of the presence of the "nucleic acid of interest" in the sample. This, however, is not how claim 284 is presently worded. This unclarity causes even more concern regarding claims such as 324 or 325 which are directed to genetic disorder detection. Clarification is requested as to what applicants mean for the metes and bounds of parts (a) and (b) regarding how the presence of the "nucleic acid of interest" is indicated in the sample versus nucleic acids that are not of interest and what signal is determinative of said presence. Do applicants mean to include some selectivity in either of parts (a) or (b) and, if so, which part or parts? This unclarity is present in all of the instantly depending claims due to their direct or indirect dependence from any of the instant independent claims as listed in the above rejected claims hereinunder.

The indefiniteness rejection is respectfully traversed.

As indicated in the opening remarks above and in the new claims above, Applicants' claimed detection process (embodied in claims 1298-1410) calls for

specifically hybridizing the oligo- or polynucleotide with the nucleic acid of interest. Applicants' other detection process (claims 1411-1472) also call for specific hybridization between the first segment (a) in the oligo- or polynucleotide provided in step (A), such first segment being "complementary to and capable of specifically hybridizing to a portion of said nucleic acid of interest." Moreover, Applicants' other claimed processes directed to aspects of chromosomal characterization (claims 1473-1581) also provide or call for specific hybridization.[10]

In view of the presentation of the new process claims, it is believed that the indefiniteness rejection has been obviated. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

The Rejection Under 35 U.S.C. §102

Claims 396 and 397 stand rejected under 35 U.S.C. §102(b) as being anticipated by Kourilsky et al.(GB 2,019,408). In the Office Action (pages 10-11), the Examiner stated that "Kourilsky et al. prepares hybridization probes made up of polynucleotides which are labeled with cytochrome C and biotin as given on page 1, line 25, through page 2, line 52, which are then utilized in hybridization assays. The instant claims rejected as listed above include any labeling location and thus are anticipated by the reference."

The anticipation rejection is respectfully traversed.

New claims 1411-1472 replaced the former and now canceled claims 396 and 397. These new claims are directed to a three-step process for a nucleic acid of interest in a sample. The first step in the process calls for (A) providing two components. First, an an oligo- or polynucleotide having two segments (i) is provided. The first segment (a) is complementary to and capable of specifically hybridizing to a portion of said nucleic acid of interest; and the second segment (b) compris s at least one protein binding nucleic acid s quence. Second, a detectable protein (ii) is provided which is capable of binding to the protein binding sequence

[10] Other of Applicants' process claims in which a detectable radioactive signal is provided by chelating c mp unds or chelating components (claims 1705-1711) similarly recite or require specific hybridization.

in the second segment of the oligo- or polynucleotide (i). In the second step (B) of the claimed process, a sample suspected of containing the nucleic acid of interest is contacted with the oligo- or polynucleotide (i) and the detectable protein (ii) to form a complex. The last step calls for (C) detecting non-radioactiveiy the presence of the protein in the complex and the nucleic acid of interest.

It is believed that the present anticipation rejection cannot be posited against the subject matter of new claims 1411-1472 for the simple and plain reason that Kourilsky et al. do not disclose nor do they suggest that their probes could or should possess a *nucleic acid sequence* that binds a detectable protein. In Applicants' claimed process, the second segment in their oligo- or polynucleotide comprises at least one protein binding nucleic acid sequence. Nowhere in Kourilsky et al. is there any mention or suggestion of a nucleic acid sequence that binds a protein. As correctly characterized by the Examiner, Kourilsky et al. prepares hybridization probes made up of polynucleotides which are labeled with cytochrome C and biotin. Kourilsky's chemically modified probes are then detected in the hybrid formed with the target through an appropriate enzyme linked with biotin. The enzyme in Kourilsky is selected according to its capacity to act on a chromagen substrate. Thus, Applicants' claimed protein binding nucleic acid sequence is altogether missing in Kourilsky's disclosure and probes, all of which are limited to protein-protein interactions.

In view of the lack of material identity between the cited Kourilsky patent and their present invention as embodied in claims 1411-1472, Applicants respectfully request reconsideration and withdrawal of the anticipation rejection.

Submission of Information Disclosure Statement

Concurrently with the filing of this Amendment, Applicants are filing a Supplemental Information Disclosure Statement for the purpose of bringing to the Examin r's attention documents cited in this and other papers submitted to the Patent Office. Being submitted in the Supplemental IDS are th following 68 documents described or listed earlier and also listed below:

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Kornberg, A., DNA Replication, W. H. Freeman And Company, San Francisco, 1980, Chapter 12, "Inhibitors of Replication," pages 415-441 [Exhibit 3]

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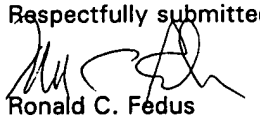
SUMMARY AND CONCLUSIONS

Claims 569-1711 have been added above for further examination on the merits. Former claims 284-568 have been canceled.

The fee for new claims 569-1711 is $24,594, based upon the $23,580 fee for the presentation of one thousand three hundred and ten (1,310) additional new claims (1,310 X $18 = $23,580) and also based upon the $1,014 fee for thirteen (13) new independent claims (13 X $78 = $1,014). As indicated in the accompanying Transmittal form, authorization is hereby given to charge the amount of $24,594 to Deposit Account No. 05-1135. This Amendment is also accompanied by a Request For An Extension Of Time (3 Months) and a Supplemental Information Disclosure Statement with authorization for the respective fees therefor. No other fee or fees are believed due. In the event that any other fee or fees are due, however, authorization is hereby given to charge the amount of any such fee(s) to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If a telephone conversation would further the prosecution of the present application, Applicants' undersigned attorney request that he be contacted at the number provided below.

Respectfully submitted,



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